XX. THE AVAILABLE CARBOHYDRATE OF FRUITS

DETERMINATION OF GLUCOSE, FRUCTOSE, SUCROSE AND STARCH

BY ELSIE MAY WIDDOWSON AND
ROBERT ALEXANDER McCANCE.

From the Biochemical Department, King's College Hospital, London.

(*Received November 24th, 1934.*)

IT is now generally recognised that determinations of available carbohydrate in fruits and vegetables are of much more value for dietetic purposes than those of total carbohydrate, whether the latter have been determined by acid hydrolysis or calculated by difference.

Figures for the available carbohydrate of the fruits and vegetables commonly eaten in this country were given by McCance and Lawrence [1929]. An acid hydrolysis was used, followed by a determination of "total" reducing power. Realising, however, that this reduction was partly due to the products from easily hydrolysable pentosans and other non-fermentable sugars, McCance and Lawrence determined these constituents separately and deducted their reducing value from that of the total sugar. The remainder they considered to be available carbohydrate. Their figures in some instances are too low because the strong acid employed and the prolonged time of boiling led to a destruction of a considerable proportion of the fructose. They mentioned that something of this sort was taking place, but considered that for practical purposes the error could be neglected.

A study of the available carbohydrates of fresh fruits has now been made by the most reliable methods which can be obtained. Sucrose, fructose, glucose and starch have each been determined separately. Since these are generally considered to be the only fruit carbohydrates which are absorbed as sugars from the gut, their sum constitutes the available carbohydrate of the fruit. Small amounts of maltose may be present in starchy fruits such as the banana. If this is the case, the error so introduced must be insignificant, since determinations of glucose and fructose by two independent methods have been found to agree. From the nature of the methods employed this would not have been the case if appreciable amounts of maltose had been present (*vide infra*).

Fresh, raw fruits only have been used, and all determinations have been carried out on the edible portion.

METHODS.

Preparation of the fruit. Almost all the fruit has been obtained from the Amalgamated Fruiterers and the Army and Navy Stores. Each variety of English and foreign fruit came from at least four, and usually from six, different sources and was known therefore to have been grown under various soil and climatic conditions. Thus a representative sample of the fruit was obtained. The analyses were all carried out during the 1934 season.

The method used for the preparation and extraction of the fruit was similar to that described by Archbold [1932] for apples. About 3 lbs. of small fruit and 6–8 lbs. of larger varieties were prepared as for eating, and the proportion of waste was determined. The edible portion was then cut up finely and well mixed. Water was determined on duplicate samples of 50 g. by drying to constant weight at 50°.

Duplicate portions of 100 g. were extracted with about 200 ml. of cold 95 % alcohol overnight. The tissue was next extracted with hot 80 % alcohol in a Soxhlet apparatus for about 16 hours, and the alcohol from the united extracts was evaporated off under reduced pressure at a temperature always below 30°. The residue was made up to 200 ml. in a graduated flask (Solution A). Free acid was determined on 20 ml. of this extract by titration with $N/10$ NaOH. Phenolphthalein was used as indicator.

Determination of reducing sugars. 50–100 ml. of Solution A were measured into a graduated 500 ml. flask, diluted with water, almost neutralised with $N/10$ NaOH and cleared with basic lead acetate and saturated sodium phosphate solutions [Archbold and Widdowson, 1931]. The solution was made up to volume and filtered. Reducing sugars were determined in this cleared filtrate (Solution B) by Lane and Eynon's [1923] copper titration method, in which methylene blue is used as an internal indicator. Dilution of Solution B was sometimes necessary before the estimations could be carried out.

Determination of sucrose. Total sugar was estimated in Solution B after inversion with 10 % citric acid for 10 mins. at 100°. The difference between the percentage of total sugar and of reducing sugar, calculated from Lane and Eynon's invert sugar table, gave the percentage of sucrose in the fruit. Preliminary tests on various fruits indicated that the values for sucrose obtained in this way were the same as those obtained after inversion with invertase (see Table I).

Table I. *Sucrose content of fruits after inversion by citric acid and by invertase.*

Results expressed as percentage in fresh material.

Fruit	Sucrose by citric acid	Sucrose by invertase
Gooseberry	0·23	0·22
Banana	4·2	4·3
Apple	2·6	2·7
Lemon	0·34	0·28
Strawberry	1·32	1·32
Tomato	0·0	0·0

Determination of the fructose/glucose ratio. Before carrying out the iodimetric procedure for the determination of the fructose/glucose ratio, it was necessary to decolorise solution B. 100 ml. were boiled with 0·5 g. B.D.H., decolorising charcoal for 1 min. and filtered. This process was repeated until the liquid was colourless. The charcoal was thoroughly washed with boiling water and the filtrate and washings made up to 200 ml. (Solution C). It has previously been shown that this procedure removes only negligible amounts of sugars from solution [Widdowson, 1931]. The iodine value of solution C was determined exactly as described by Archbold and Widdowson [1931]. The fructose/glucose ratio was calculated by solving the simultaneous equations obtained from the iodimetric determination and from the estimation of the reducing power of solution B by Lane and Eynon's copper method.

Fructose has also been determined directly in Solution B in a number of different fruits by the method described by Oppel [1930]. This is a colorimetric method based upon the colour developed when fructose is heated with diphenylamine and strong hydrochloric acid. Since the acid hydrolyses any sucrose present, the value obtained for fructose is the sum of the free fructose and the fructose derived from the sucrose. A standard solution of sucrose was used for the comparisons. Table II shows the amounts of fructose (including free fructose and sucrose-fructose) in a variety of fruits as estimated by the two independent methods.

Table II. *Total fructose in fresh fruits estimated by Fehling's solution and iodine and by diphenylamine.*

Results expressed as percentage of fructose in fresh fruit.

Fruit	Fehling's solution and iodimetric method	Diphenylamine method
Pear	7·9	7·7
Blackberry	3·6	3·6
Grapefruit	2·3	2·6
Pineapple	4·9	5·0
Orange	4·0	4·1
Grape	8·0	7·9
Cranberry	0·8	1·1
Date	29·9	29·4
Banana	6·9	7·0

It will be observed that in most cases agreement is very close. In the case of cranberries the slightly higher results given by the diphenylamine method can be explained by the fact that in this fruit there is a large excess of glucose with which the reagent reacts to a small extent.

Determination of starch. The fresh fruit was in every case tested with iodine in order to detect the presence of starch. Of all the fruits examined, starch was found to be present only in the banana, apple, pear and tomato. The residue remaining after alcoholic extraction of these fruits was dried at 100° overnight and weighed.

The dried residue from the banana was ground up in a mortar and sifted. Duplicate samples of 0·5 g. were weighed out and soaked in 10 ml. water for several hours. It was impossible to grind the residues from the apple, pear and tomato owing to their tough and leathery nature. These were therefore weighed out without grinding and soaked in water as just described. The wet material in each case was ground up in a mortar to a fine pulp and transferred quantitatively to a small flask with a further 40 ml. of water, which was then boiled.

Takadiastase was used to hydrolyse the starch, and the reducing power was determined on the filtrate after clearing with basic lead acetate by Hanes's [1929] modification of the Hagedorn and Jensen ferricyanide technique. Full details have been described by Widdowson [1932].

Results.

The results of the analyses are shown in Table III.

Values are given for the percentages of glucose, fructose, sucrose and starch in the edible portion of the fruits, and the total available carbohydrate is calculated as the sum of these 4 individual carbohydrates. The water content of the fruits is also shown and their titratable acidity. For each fruit a factor is also given. By

Table III. *Available carbohydrate content of fruits.*

Fruit	Percentage in edible portion: Glucose	Fructose	Sucrose	Starch	Total available carbohydrate	Water	Titratable acidity ml. $N/10$ NaOH ≡ 100 g. fruit	Conversion factor	Nature of edible (analysed) portion and waste
Apples, Empire eating	2·20	7·25	2·70	0·02	12·17	84·1	67	0·75	Flesh analysed. Skin and "core" discarded
Apples, English eating	1·72	6·08	3·62	0·30	11·72	84·5	82	0·79	Flesh analysed. Skin and "core" discarded
Apples, English cooking	1·82	5·01	2·40	0·35	9·58	85·6	189	0·81	Flesh analysed. Skin and "core" discarded
Apricots	1·93	0·37	4·35	0	6·65	86·6	230	0·92	Flesh and skin analysed. Stones discarded
Bananas	5·82	3·78	6·58	3·02	19·20	70·7	68	0·57	Flesh analysed. Skin discarded as waste
Blackberries, mixed hedge and cultivated	3·24	2·88	0·24	0	6·36	82·0	139	1·0	Whole fruit analysed
Cherries, eating	4·70	7·24	0	0	11·94	81·5	113	0·87	Flesh and skin analysed. Stalks and stones discarded
Cherries, cooking	5·50	6·11	0	0	11·61	79·8	145	0·84	Flesh and skin analysed. Stalks and stones discarded
Cranberries	2·66	0·74	0·14	0	3·54	87·0	389	1·0	Whole fruit analysed
Currants, black	2·35	3·67	0·62	0	6·64	77·4	443	0·98	Flesh, skin and pips analysed. Stalks discarded
Currants, red	2·28	1·93	0·15	0	4·36	82·8	350	0·97	Flesh, skin and pips analysed. Stalks discarded
Currants, white	3·03	2·56	0	0	5·59	83·3	223	0·96	Flesh, skin and pips analysed. Stalks discarded
Damsons	5·22	3·42	0·96	0	9·60	77·5	307	0·90	Flesh and skin analysed. Stones and stalks discarded
Dates	32·00	23·70	8·20	0	63·90	14·6	65	0·86	Flesh and skin analysed. Stones discarded
Figs, green	5·54	4·00	0	0	9·54	84·6	50	0·98	Flesh, skin and pips analysed. Stalks discarded
Gooseberries, green	1·47	1·70	0·25	0	3·42	89·9	237	0·99	Flesh, skin and pips analysed. "Tops and tails" discarded
Gooseberries, ripe	4·40	4·10	0·71	0	9·21	83·71	245	0·99	Flesh, skin and pips analysed. "Tops and tails" discarded
Grapes, black	8·20	7·28	0	0	15·48	80·7	96	0·81	Flesh analysed. Skin, pips and stalks discarded
Grapes, white	8·12	8·01	0	0	16·13	79·3	58	0·95	Flesh and skin analysed. Stalks and pips discarded
Grapefruit	1·95	1·24	2·14	0	5·33	90·7	203	0·48	Flesh only analysed. Skin, pith and pips discarded
Greengages	5·00	2·60	4·16	0	11·76	78·2	219	0·95	Flesh and skin analysed. Stones and stalks discarded
Lemons, whole	1·40	1·35	0·41	0	3·16	85·2	213	1·0	Whole fruit analysed including skin
Lemon juice	0·52	0·92	0·18	0	1·62	91·3	947	0·36	Juice only analysed
Loganberries	1·94	1·26	0·23	0	3·43	85·0	332	1·0	Whole fruit analysed
Melons, Cantaloupe	1·16	0·83	3·26	0	5·25	93·6	17	0·59	Flesh analysed. Skin and pips discarded
Melons, yellow	2·09	1·52	1·43	0	5·04	94·2	19	0·59	Flesh analysed. Skin and pips discarded
Mulberries	4·44	3·64	0	0	8·08	85·0	247	1·0	Whole fruit analysed
Oranges	2·51	1·81	4·19	0	8·51	86·1	102	0·75	Flesh only analysed. Outer skin and pips discarded
Orange juice	2·36	2·38	4·70	0	9·44	87·7	134	0·46	Juice only analysed
Peaches	1·47	0·93	6·66	0	9·06	86·7	114	0·87	Flesh and skin analysed. Stones discarded
Pears, Empire eating	3·47	6·28	1·04	0	10·79	83·0	25	0·69	Flesh only analysed. Skin and "core" discarded
Pears, English eating	2·44	7·00	0·98	0	10·42	83·4	44	0·75	Flesh only analysed. Skin and "core" discarded
Pears, English cooking	2·18	6·00	1·12	0·04	9·34	83·0	57	0·77	Flesh only analysed. Skin and "core" discarded
Pineapple, fresh	2·32	1·42	7·89	0	11·63	84·3	169	0·54	Flesh analysed. Skin and "core" discarded
Plums, dessert	4·00	1·34	4·26	0	9·60	84·1	252	0·95	Flesh and skin analysed. Stones and stalks discarded
Plums, cooking	3·50	1·27	1·45	0	6·22	85·1	356	0·91	Flesh and skin analysed. Stones and stalks discarded
Pomegranate juice	5·46	6·14	0	0	11·60	85·4	146	0·56	Juice only analysed
Raspberries	2·26	2·39	0·96	0	5·61	83·2	275	1·0	Whole fruit analysed
Strawberries	2·59	2·32	1·30	0	6·21	88·9	125	0·97	Flesh and pips analysed. Stalks discarded
Tomatoes	1·63	1·17	0	0·02	2·82	93·4	73	0·99	Flesh, skin and pips analysed. Stalks discarded

multiplying the percentages in the edible portion by the factor, the quantities which would be obtained from the food as purchased can be derived. The final column shows the parts of the fruits which were included in the edible portion.

Discussion.

It will be noticed that only a very small proportion of the ripe fruits contains starch, and even in these cases the amount present is negligible. Bananas are exceptional. 16 % of the total available carbohydrate was present as starch in the samples examined, *i.e.* 3 % of starch in the fresh fruit. It is a well-known fact that the starch/sugar ratio in the banana depends upon the degree of ripeness of the fruit, and quite different results would no doubt have been obtained had unripe or over-ripe fruits been analysed.

Most of the fruits examined contain approximately equal amounts of free fructose and glucose, but apples and pears have much more fructose than glucose, and plums, damsons, apricots and similar fruits and also cranberries contain more free glucose than fructose. Grapes contain glucose and fructose in nearly equal amounts.

A few fruits, notably cherries, grapes, figs, tomatoes, pomegranates and mulberries, contain no sucrose at all. In one or two cases, *e.g.* apricots and peaches, sucrose constitutes the greater part of the total sugar present.

Comparison of the present results with those obtained after acid hydrolysis.

It has been stated earlier in the paper that McCance and Lawrence's [1929] figures for the carbohydrate contents of fruits were too low, owing to a destruction of part of the fructose during the acid hydrolysis. This is shown to be the case by the fact that on boiling a 2 % solution of fructose with hydrochloric acid (5 % by volume), 28 % of the total fructose present was destroyed during the first hour and 48 % after 2 hours. Glucose was almost unchanged by this treatment.

Samples of seven of the fruits used in the present investigation were boiled with 5 % acid for 2 hours and estimated as described by McCance and Lawrence. The results of these determinations are shown in Table IV, Column 1. Column 2

Table IV. *Comparison of available carbohydrate in fruits as determined in the present investigation and after acid hydrolysis.*

Results expressed as percentage of edible portion of fresh fruit.

Fruit	Direct determination of carbohydrate after acid hydrolysis	Pentose	Carbohydrate corrected for pentose	Carbohydrate corrected for pentose and fructose	Sum of glucose, fructose, sucrose and starch
Blackberries*	8·4	1·8	6·6	8·4	7·6
Cranberries	4·0	0·9	3·1	3·5	3·5
Damsons	9·5	2·2	7·3	9·3	9·6
Grapes, black	12·5	0·6	11·9	15·5	15·5
Grapefruit	5·3	1·1	4·2	5·3	5·3
Orange juice	6·9	0	6·9	9·2	9·4
Pears, cooking	7·9	1·7	6·2	9·5	9·3

* Blackberries have been analysed on 3 occasions. The figure in Table III is the mean of all three analyses, while the figure in this table is the result of one of them.

shows the pentose content of fruits and Column 3 the available carbohydrate after deducting the pentose sugars [McCance and Lawrence, 1929]. Taking the figures for fructose found in the present investigation (free fructose + sucrose-fructose), and assuming that 50 % of this had been destroyed during the 2 hours' hydrolysis, the figures in Column 3 have been corrected, and the results are shown in Column 4. It will be seen that in almost every case they agree very closely with the present results for total available carbohydrate calculated as the sum of the glucose, fructose, sucrose and starch (Column 5). It is evident therefore, that McCance and Lawrence were justified in deducting the values for pentose sugars, and that their figures for vegetables which contain no fructose were probably correct. Further, McCance and Lawrence's published results were not so much too low as the differences between Columns 2 and 4 suggest, since they took the mean of a 1- and 2-hour hydrolysis, and the present figures were obtained after treating for a full 2 hours with the acid.

Summary.

The glucose, fructose, sucrose and starch in 41 varieties of fruit have been determined.

Sucrose was estimated by the increase in reducing power after inversion.

The glucose/fructose ratio was calculated by solving the simultaneous equations obtained from an iodimetric determination and from the estimation of reducing power by Fehling's solution. Fructose was also determined by diphenylamine with concordant results.

Starch was estimated as glucose and maltose after digestion with takadiastase.

The sum of these four individual carbohydrates was taken to represent the available carbohydrate of the fruit.

Part of the expenses of the investigation has been defrayed by the Medical Research Council. One of us (E. M. W.) is indebted to the Medical Research Council for a part-time grant.

References.

Archbold (1932). *Ann. Bot.* **46**, 407.
—— and Widdowson (1931). *Biochem. J.* **25**, 101.
Hanes (1929). *Biochem. J.* **23**, 99.
Lane and Eynon (1923). *J. Soc. Chem. Ind.* **42**, 132 T.
McCance and Lawrence (1929). *Med. Res. Coun. Special Report, Series.* No. 135.
Oppel (1930). *Biochem. Z.* **229**, 85.
Widdowson (1931). *Biochem. J.* **25**, 863.
—— (1932). *Ann. Bot.* **46**, 597.